Skoltech
Skolkovo Institute of Science and Technology

# Jury Member Report – Doctor of Philosophy thesis.

**Name of Candidate:** Alexander Martynov

**PhD Program:** Life Sciences

**Title of Thesis:** Using mathematical modeling to understand prokaryotic adaptive immunity

**Supervisor:** Prof. Konstantin Severinov

**Co-Supervisor:** Prof. Jaroslav Ispolatov

**Chair of PhD defense Jury:** Prof. Mikhail Gelfand ***Email**: m.gelfand@skoltech.ru*

**Date of Thesis Defense:** 25 October 2018

**Name of the Reviewer: Edze Westra**

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|---|---|
| I confirm the absence of any conflict of interest<br>yes<br>(Alternatively, Reviewer can formulate a possible conflict) | **Signature:**<br>  <br>**Date: 26-09-2018** |

*The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.*

*If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.*

**Reviewer's Report**

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international

level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

- Brief evaluation of the thesis quality and overall structure of the dissertation.

The thesis of Alexander Martynov entitled "Using Mathematical Modeling To Understand Prokaryotic Adaptive Immunity" applies a combination of modeling and experiments to examine how expression levels and binding kinetics of CRISPR-Cas surveillance complexes to MGE genomes can lead to partial immunity phenotypes, both in the context of bacteria-phage and bacteria-plasmid interactions, and how this can shape selection for specific CRISPR array lengths in the context of single or mixed phage infections.

What I liked about the thesis is that it explores how macroscopic processes (i.e. resistance phenotypes of individual bacteria and survival probabilities of phage and plasmids) are shaped by the kinetics of the underlying molecular processes – including phage/plasmid replication (MGE birth) and CRISPR-target interactions (MGE death). Using this approach, Alexander Martynov gets a handle on determinants of resistance levels, selection for CRISPR array lengths and persistence of MGEs in presence and absence of their evolution.

I also feel there is scope for improvement of the thesis.

1. The introduction chapter covers a lot of ground, but in doing so, becomes somewhat unfocussed and lacks synthesis. I would recommend a more concise introduction that provides a clearer vision of the state of the field and the open questions that this thesis aims to address.
2. A more critical reflection and - where possible – explicit test is needed of how robust the model predictions are to the underlying assumptions. In Chapter 3, I am concerned about the assumption of constant time interval between spacer acquisitions. In nature selection may act to increase the rate of spacer acquisition. This would result in longer arrays with more spacers that are functional (i.e. against which the phage has not yet evolved escape), and is likely to be favoured by natural selection in the face of an evolving phage. I feel this aspect is insufficiently explored and/or discussed in the thesis.
3. In Chapter 4, experiments show that CRISPR ON cells within a colony are heterogeneous with regards to whether or not they retain the target plasmid (only ± 1 in 4000 cells within a colony carries the plasmid). To me, the reasons for this are not intuitive, and more should be done to clarify how this observation is explained by the model.

- The relevance of the topic of dissertation work to its actual content

I'm unclear on this point, since in my opinion the topic of the dissertation is determined by its content.

- The relevance of the methods used in the dissertation

Alexander Martynov uses a powerful combination of modeling and experiments to explore questions that are highly relevant to understanding CRISPR-MGE interactions.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art

Chapter 3 has already been published in a high-impact journal (PLoS Comp Biol) and Chapter 4 is of publication quality (although I have some comments on both chapters).

- The relevance of the obtained results to applications (if applicable)

This research sits in the area of fundamental science, but the insights could be used to optimize CRISPR-mediated protection of bacterial strains in various applications (e.g. fermentations in industry).

- The quality of publications

Chapter 3 has been published in a high-impact journal (PLoS Comp Biol), and comprises a solid piece of work, although I would like Alexander Martynov to explore in somewhat more detail how the assumptions underlying the model will impact the model predictions, particularly with regards to the selection for CRISPR array length, as explained above.

The summary of issues to be addressed before/during the thesis defense

Major points:

1. A more critical reflection and - where possible – explicit test would be needed of how robust the model predictions are to the underlying assumptions. In Chapter 3, I am concerned about the assumption of constant time interval between spacer acquisitions. In nature selection may act to increase the rate of spacer acquisition. This would result in longer arrays with more spacers that are functional (i.e. against which the phage has not yet evolved escape), and is likely to be favoured by natural selection in the face of an evolving phage. I feel this aspect is insufficiently explored and/or discussed in the thesis.
2. In Chapter 4, experiments show that CRISPR ON cells within a colony are heterogeneous with regards to whether or not they retain the target plasmid (only ± 1 in 4000 cells within a colony carries the plasmid). To me, the reasons for this are not intuitive, and more should be done to clarify how this observation is explained by the model. Is the second equilibrium not stable ?
3. Primer efficiencies for qPCR are outside of the commonly accepted 0.8-1.2 range. Please speficify / discuss how this may influence the results.

Minor points:

1. The introduction chapter covers a lot of ground, but in doing so, becomes somewhat unfocussed and lacks synthesis. I would recommend a more concise introduction that provides a clearer vision of the state of the field and the open questions that this thesis aims to address.
2. Page 14 talks about the mechanism of primed spacer acquisition. Apart from the kinetic explanation (i.e. rapid destruction of spacer substrates), selection will also be important (stronger selection in absence of full resistance) – please adjust accordingly.
3. Page 16; this is supported by the fact that there are conserved spacers …. Narrative assumes that conservation is driven by selection (i.e. that this is adaptive). Not necessarily the case, as shown in various models on conservation of trailer end ; could be due to drift or hitch-hiking effects – please adjust.
4. Page 20; Section on Abi feels out of context
5. Page 29 – while natural virus-host populations seem to coevolve… do they? Please provide refs of some examples where they do, but also mention that coevolution is very often not detected.
6. Page 30 – Overall the evolutionary models… The justification of the work presented in the thesis can be made stronger by pointing out the specific questions that previous models have asked, and how the questions that are addressed in this thesis differ.
7. Page 36 – (rather than…) distinction is unclear to me, please explain more
8. Page 37 – make more explicit what the likely drivers are (according to this model) of observed variation in CRISPR array length in natural populations.
9. Page 40 – model assumes fixed expression level of CRISPR-Cas system, whereas this would appear a strong target of natural selection ; please discuss / explore.
10. Page 41 - Assumption of constant CRISPR array length at odds with experimental evolution studies ; please discuss.

11. Chapter 3 should include a table with all the model parameters.
12. In Chapter 3 a clearer discussion is needed about spacer retainment vs acquisition rate (related to major comment 1). This point is only briefly mentioned in the discussion, but should be expanded by textual changes or – if possible – further exploration of the model predictions when these assumptions are relaxed.
13. When discussing social implications of CRISPR, reference should be made to the "herd immunity" paper in ELife by the Bollback group, and perhaps van Houte Nature 2016 (see SI).
14. In introducing Chapter 4, please discuss in detail PMID: 29717009
15. Page 75 – bottleneck-like effect ; I suggest rephrasing as I personally found this term from population genetics confusing in this context (since you are not looking at population genetics of the plasmid – if anything, you show that plasmid evolution does not play a role in the observed effects of plasmid persistence).
16. Page 75 – bimodality in cellular distribution ; this is not something CRISPR-specific, so I suggest rephrasing this accordingly.
17. Page 75 – "treacherous" ; please rephrase.
18. Page 78/79; as often done… please add refs.
19. Please adjust order of text in legend Fig. 4.5 .
20. Section 4.3.3 was hard to follow and would benefit from textual changes to improve the clarity of the narrative and experimental design.
21. Page 94 – (Ref) – please insert the ref.
22. Page 95 – "restreaking" – replating ?

**Provisional Recommendation**

X *I recommend that the candidate should defend the thesis by means of a formal thesis defense*

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*